

Unsaturated phosphinic analogues of γ -aminobutyric acid as GABA_C receptor antagonists

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Abstract

The phosphinic and methylphosphinic analogues of γ -aminobutyric acid (GABA) are potent GABA_C receptor antagonists but are even more potent as GABA_B receptor agonists. Conformationally restricted unsaturated phosphinic and methylphosphinic analogues of GABA and some potent GABA_B receptor phosphonoamino acid antagonists were tested on GABA_C receptors in *Xenopus* oocytes expressing human retinal ρ_1 mRNA. 3-Aminopropyl-*n*-butyl-phosphinic acid (CGP36742), an orally active GABA_B receptor antagonist, was found to be a moderately potent GABA_C receptor antagonist (IC₅₀ = 62 μ M). The unsaturated methylphosphinic and phosphinic analogues of GABA were competitive antagonists of the GABA_C receptors, the order of potency being [(*E*)-3-aminopropen-1-yl]methylphosphinic acid (CGP44530, IC₅₀ = 5.53 μ M) > [(*E*)-3-aminopropen-1-yl]phosphinic acid (CGP38593, IC₅₀ = 7.68 μ M) > [(*Z*)-3-aminopropen-1-yl]methylphosphinic acid (CGP70523, IC₅₀ = 38.94 μ M) > [(*Z*)-3-aminopropen-1-yl]phosphinic acid (CGP70522, IC₅₀ > 100 μ M). This order of potency differs from that reported for these compounds as GABA_B receptor agonists, where the phosphinic acids are more potent than the corresponding methylphosphinic acids. © 1997 Elsevier Science B.V.

Keywords: GABA_C receptor; GABA (γ -aminobutyric acid); GABA_B receptor agonist; GABA_B receptor antagonist; *Xenopus* oocyte; *trans*-4-Aminocrotonic acid; *cis*-4-Aminocrotonic acid

1. Introduction

γ -Aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the central nervous system (CNS) and activates three major subtypes of GABA receptors, the GABA_A, GABA_B and GABA_C receptors. GABA_A receptors are ligand-gated Cl⁻ channels which are inhibited by the alkaloid, bicuculline (Johnston, 1996a). These are heterooligomeric receptors made up of α , β , γ , and δ subunits. GABA_B receptors are transmembrane receptors coupled to second messenger systems and Ca²⁺ and K⁺ channels via G-proteins. These receptors are not blocked by bicuculline but are activated by (–)-baclofen and 3-aminopropylphosphinic acid (CGP27492) and blocked by phaclofen and saclofen (Kerr and Ong, 1995).

GABA_C receptors (sometimes called GABA_{NANB} and ρ receptors) were first proposed when a series of confor-

mationally restricted GABA analogues, including *cis*-4-aminocrotonic acid (CACA), that had bicuculline-insensitive depression actions on neuronal activity, showed no affinity for [³H]baclofen binding sites in rat cerebellar membranes (Drew et al., 1984). GABA_C receptors with similar pharmacology were first found in neurons from rat retina (Feigenspan et al., 1993) and white perch retina (Qian and Dowling, 1993). In rat retina, rod bipolar cells contain bicuculline-insensitive, baclofen-insensitive receptors that were activated by CACA (Feigenspan et al., 1993). These were detected by the co-application of GABA with 100 μ M bicuculline to abolish the GABA_A component (Feigenspan et al., 1993). In white perch retina, rod-driven horizontal cells (H4) and not bipolar cells showed GABA_C receptor-like pharmacology. Application of GABA on bipolar cells showed rapid desensitisation while on rod-driven horizontal cells, desensitisation was not observed (Qian and Dowling, 1993). Subsequently, GABA_C receptors were found on cone-driven horizontal cells in catfish (Dong et al., 1994) and bipolar terminals in tiger salamander (Lukasiewicz et al., 1994).

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The expression of mRNA from bovine retina into *Xenopus* oocytes showed that GABA activated two distinct GABA receptors. Both receptors activated Cl^- currents. One was mediated by GABA_A receptors and was blocked by bicuculline and the other was mediated by GABA_C receptors and was insensitive to both bicuculline and baclofen (Polenzani et al., 1991). Subsequently, two cDNAs that have 30–38% sequence identity with GABA_A receptor subunits were cloned from human retinal mRNA (Cutting et al., 1991, 1992). These subunits have been termed ρ_1 and ρ_2 and have 74% sequence identity (Cutting et al., 1991, 1992).

The species equivalent of the human ρ_1 and ρ_2 subunits have been cloned from rat (Enz et al., 1995). These show 88–99% homology with the respective human sequences. The use of PCR and in situ hybridisation have shown high expression of both the ρ_1 and ρ_2 subunits in rod bipolar cells. However, only the ρ_2 subunit is expressed in the CNS, particularly in the hippocampus and cortex (Enz et al., 1995). Recently, a third ρ subunit was cloned from rat retina cDNA (Ogurusu and Shingai, 1996). This subunit exhibits 63% and 61% sequence homology to the human ρ_1 and rat ρ_2 sequences, respectively (Ogurusu and Shingai, 1996).

Expression of human ρ subunits in *Xenopus* oocytes generates homooligomeric GABA receptors with intrinsic Cl^- channels. These receptor ion channels are activated by GABA and CACA but are insensitive to both bicuculline, (–)-baclofen, barbiturates and benzodiazepines. They have been shown to be sensitive to picrotoxin and have been classified as GABA_C receptors (Cutting et al., 1991, 1992; Polenzani et al., 1991; Shimada et al., 1992; Kusama et al., 1993a,b; Wang et al., 1994; Bormann and Feigenspan, 1995; Johnston, 1996b).

The most potent GABA_C receptor agonists are *trans*-4-aminocrotonic acid (TACA, $K_d = 0.6 \mu\text{M}$) and GABA ($K_d = 1.7 \mu\text{M}$) (Woodward et al., 1993). TACA, a conformationally restricted analogue of GABA in an extended conformation, is also a GABA_A receptor agonist (Johnston, 1996a). CACA, a conformationally restricted analogue of GABA in a folded conformation, has moderate partial agonist activity at GABA_C receptors ($K_d = 74 \mu\text{M}$) and may be the most selective agonist for this receptor subtype (Johnston, 1996b). A series of GABA analogues were tested for agonist and antagonist activity at GABA_C receptors using poly(A)⁺ RNA from mammalian retina injected in *Xenopus* oocytes (Woodward et al., 1993). Several potent GABA_C receptor antagonists were identified including (3-aminopropyl)methylphosphinic acid (CGP35024; $K_B = 0.8 \mu\text{M}$), 3-aminopropylphosphinic acid (CGP27492; $K_B = 1.8 \mu\text{M}$), and 3-aminopropylphosphonic acid (3-APA, $K_B = 10 \mu\text{M}$) (Woodward et al., 1993). CGP35024 and CGP27492 are not selective for GABA_C receptors as they are also very potent GABA_B receptor agonists while 3-APA is a GABA_B receptor antagonist. A more recently synthesised compound,

1,2,5,6-tetrahydropyridine-4-yl)methylphosphinic acid (TPMPA), does show potent and selective GABA_C receptor antagonist activity ($K_d = 2.1 \mu\text{M}$) (Murata et al., 1996; Ragozzino et al., 1996). In this study, we demonstrate that the phosphinic and methylphosphinic acid derivatives of CACA and TACA, and 3-aminopropyl-*n*-butyl-phosphinic acid (CGP36742), an orally active GABA_B receptor antagonist, are GABA_C receptor antagonists (Bittiger et al., 1992, 1993; Froestl et al., 1992, 1995a; Olpe et al., 1993).

2. Materials and methods

2.1. Materials

[(*E*)-3-Aminopropen-1-yl]methylphosphinic acid (CGP44530), [(*E*)-3-aminopropen-1-yl]phosphinic acid (CGP38593), [(*Z*)-3-aminopropen-1-yl]methylphosphinic acid (CGP70523), [(*Z*)-3-aminopropen-1-yl]phosphinic acid (CGP70522), 3-aminopropyl-*n*-butyl-phosphinic acid (CGP36742), 3-aminopropyl(diethoxymethyl)phosphinic acid (CGP35348), 3-aminopropyl(cyclohexylmethyl)phosphinic acid (CGP46381), (2*S*)-3-amino-2-hydroxypropyl(cyclohexylmethyl)phosphinic acid (CGP51176) and (2*R*,1'*S*)-(3-*N*-[1'(3,4-dichlorophenyl)ethyl]amino-2-hydroxypropyl)benzylphosphinic acid (CGP55845A) were synthesised previously by Froestl et al. (1992, 1995a,b). CACA and TACA were prepared as previously described (Johnston et al., 1975) by Dr. K.N. Mewett (Department of Pharmacology, The University of Sydney, Sydney, Australia). GABA was purchased from Sigma (St. Louis, MO, USA).

2.2. Electrophysiological recording

Human ρ_1 cDNA in pcDNA (Invitrogen, San Diego, CA, USA) was obtained from Dr. George Uhl (National Institute for Drug Abuse, Baltimore, MD, USA). The plasmid was linearized with *Xba*I and cRNA made using the 'Mmessage Mmachine' kit from Ambion (Austin, TX, USA). 50 ng of cRNA was injected into defolliculated Stage V *Xenopus* oocytes. Two to seven days later, receptor activity was measured by two-electrode voltage-clamp recording using a Geneclamp 500 amplifier (Axon Instruments, Foster City, CA, USA) and a MacLab 2e recorder (ADInstruments, Sydney, NSW, Australia). Oocytes were voltage clamped at -60 mV and continuously superfused with ND96 buffer (96 mM NaCl, 2 mM KCl, 1.8 mM CaCl_2 , 1 mM MgCl_2 and 5 mM HEPES, pH 7.5). For receptor activation measurements, the indicated concentrations of agonist and antagonist were added to ND96.

2.3. Analysis of kinetic data

Current (I) as a function of agonist concentration ($[A]$) was fitted by least squares to $I = I_{\text{max}} [A]^{n_H} / (\text{EC}_{50}^{n_H} +$

$[A]^{n_H}$), where I_{max} is the maximal current, the EC_{50} is the effective dose that activates 50% of the maximal current and n_H is the Hill coefficient. EC_{50} values are expressed as mean \pm S.E.M. ($n = 3-6$) and are determined by fitting data from individual oocytes using Kaleidagraph 2.1 (1990). Current (I) as a function of antagonist concentration ($[Ant]$) was fitted by least squares to $I = I_{max} - \{I_{max} [Ant]^{n_H} / (IC_{50}^{n_H} + [Ant]^{n_H})\}$, where the IC_{50} is the inhibition dose that blocks 50% of the current generated by 1 μ M GABA and n_H is the Hill coefficient. IC_{50} values are expressed as mean \pm S.E.M. ($n = 3-6$). K_B values are the apparent dissociation constants for the antagonists and were determined using Schild plot analysis (Arunlakshana and Schild, 1959). $-\log K_B$ values were determined using the following equation: $\log \{(A)/(A^*) - 1\} = m \log [Ant] - \log K_B$, where A is the EC_{50} of GABA in the presence of a known antagonist concentration, A^* is the EC_{50} of GABA in the absence of an antagonist, $[Ant]$ is the concentration of the antagonist, and m is the slope of the curve. For simple competitive antagonism, m is 1. $-\log K_B$ values were determined by fitting data to the above function using Kaleidagraph 2.1 (1990). Schild analyses were carried out for compounds that had IC_{50} values of less than 30 μ M.

3. Results

Expression of human ρ_1 mRNA in *Xenopus* oocytes generated $GABA_C$ receptors which showed a dose-depen-

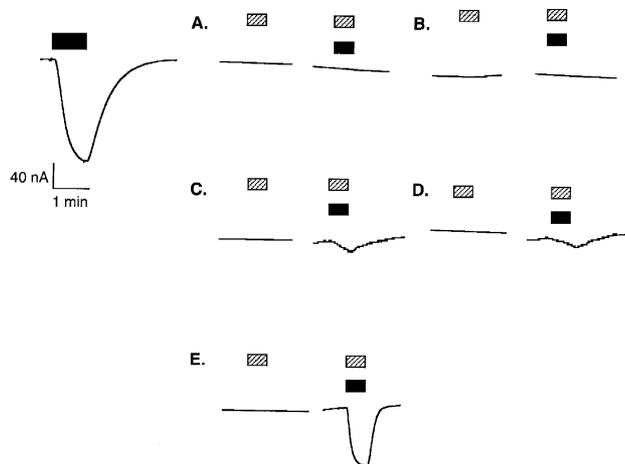


Fig. 1. Expression of human ρ_1 receptors in *Xenopus* oocytes produce homooligomeric $GABA_C$ receptors with intrinsic Cl^- channels. GABA (1 μ M) activates the Cl^- channels (duration indicated by filled bar) and produces an inward current when the oocyte is clamped at -60 mV. (A) CGP38593 (100 μ M), (B) CGP44530 (100 μ M), (C) CGP70523 (100 μ M), (D) CGP36742 (100 μ M), and (E) CGP70522 (300 μ M) do not activate the receptor (duration indicated by hatched bar). However, when (A) CGP38593 (100 μ M), (B) CGP44530 (100 μ M), (C) CGP70523 (100 μ M), (D) CGP36742 (100 μ M), and (E) CGP70522 (300 μ M) are co-applied with GABA (1 μ M), the GABA response is blocked or reduced.

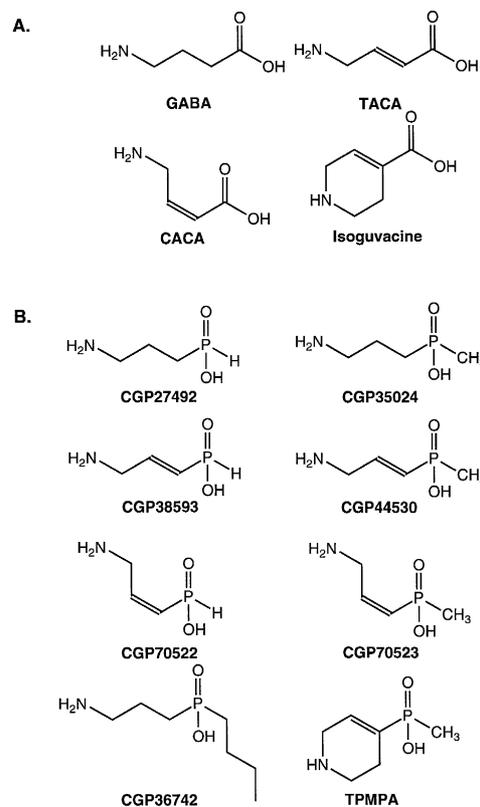


Fig. 2. (A) Structures of compounds that show agonist activity at $GABA_C$ receptors. (B) Structures of compounds that show antagonist activity at $GABA_C$ receptors.

dent GABA-activated inward current when the cell was voltage clamped at -60 mV. This could be blocked by compounds such as CGP44530, CGP38593, CGP70523, CGP70522 and CGP36742 as shown in Fig. 1. The structures of the compounds are shown in Figs. 2 and 3. These compounds were first screened at 100 μ M to determine agonist activity, by activation of Cl^- channels, or antagonist activity, by blocking the activation of the channels by 1 μ M GABA. Fig. 2 shows the active compounds that had some effect at 100 μ M as agonists (Fig. 2A) or antagonists (Fig. 2B) at $GABA_C$ receptors and Fig. 3 shows the compounds that had no effect at 100 μ M as agonists or antagonists at $GABA_C$ receptors. Only the carboxylic acids, TACA, GABA and CACA activated the Cl^- channel. TACA was more potent than GABA with an EC_{50} of 0.44 ± 0.02 μ M and was almost a full agonist with a maximal TACA dose generating 95% of the maximal GABA-activated current. GABA was found to have an EC_{50} value of 0.82 ± 0.09 μ M. CACA was less potent than GABA. The EC_{50} was 37.4 ± 6.1 μ M and was a partial agonist with a maximal CACA dose generating 75% of the maximal GABA-activated current (Table 1). The Hill coefficients (n_H), as shown in Tables 1 and 2, were greater or equal to 2 which suggests that more than one molecule of the agonist is required to bind before the Cl^- channels can open. These findings are in agreement with those of Woodward et al. (1993).

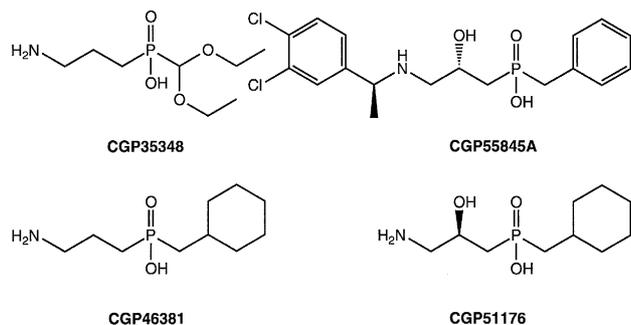


Fig. 3. Structures of orally active GABA_B receptor antagonists with no cognitive enhancement effects. These compounds show no affinity for GABA_C receptors as either agonists or antagonists when tested at 100 μ M.

CGP35024, CGP27492, CGP44530, CGP38593, CGP70523 and CGP70522 did not activate any current on their own (Fig. 1). They acted as GABA_C receptor antagonists, inhibiting the current activated by 1 μ M GABA (Fig. 1). IC₅₀ values were obtained for these compounds (Table 1) and Schild analyses were carried out for the active compounds (Table 2). K_B (binding constant) values for CGP27492, CGP44530, CGP35024, and CGP38593 are shown in Table 1.

The methylphosphinic analogue, CGP44530, and phosphinic analogue, CGP38593, of TACA were antagonists with IC₅₀ values of 5.5 ± 1.2 μ M and 7.7 ± 0.7 μ M respectively. These compounds had lower affinity for the GABA_C receptor expressed in *Xenopus* oocytes than the corresponding methylphosphinic analogue, CGP35024, and phosphinic analogue, CGP27492, of GABA. CGP35024 had an IC₅₀ of 0.75 ± 0.07 μ M and CGP27492 had an

Table 1
Summary of EC₅₀, IC₅₀, K_B and Hill coefficients of various agonists and antagonists at the GABA_C receptor expressed in *Xenopus* oocytes

	EC ₅₀ (μ M)	IC ₅₀ (μ M)	n_H	K_B (μ M)
GABA	0.82 ± 0.09		2.6 ± 0.2	
TACA	0.44 ± 0.02		2.4 ± 0.2	
CACA	37.4 ± 6.1		2.2 ± 0.3	
Isoguvacine ^a	99			
CGP35024		0.75 ± 0.07	1.8 ± 0.1	0.58 ± 0.14
CGP44530		5.5 ± 1.2	2.4 ± 0.5	8.6 ± 1.6
CGP70523		38.9 ± 4.9	1.6 ± 0.1	
CGP27492		2.47 ± 0.04	1.9 ± 0.2	3.2 ± 1.0
CGP38593		7.7 ± 0.7	1.8 ± 0.4	15.5 ± 1.7
CGP70522		> 100		
CGP36742		62.5 ± 0.5	3.0 ± 0.4	
TPMPA ^a				2.1

EC₅₀ is the effective dose that activates 50% of the maximal current when tested at ρ_1 receptors expressed in *Xenopus* oocytes. IC₅₀ is the concentration that inhibits 50% of the response produced by 1 μ M GABA. Data are the mean \pm S.E.M. ($n = 3-6$ oocytes). n_H is the Hill coefficient. K_B is the binding constant for the antagonist. These were determined using Schild plot analysis assuming competitive antagonism over the tested concentrations (Table 2).

^a Data from Murata et al. (1996).

Table 2

Results of Schild analyses of CGP35024, CGP27492, CGP44530 and CGP38593 at the GABA_C receptor expressed in *Xenopus* oocytes

Antagonist (μ M)	EC ₅₀ of GABA (μ M)	n_H	Slope of Schild plot
CGP35024			
3	4.5 ± 0.1	2.3 ± 0.1	1.14
10	10.0 ± 1.4	2.2 ± 0.2	
30	28.8 ± 4.2	1.9 ± 0.2	
CGP27492			
10	3.2 ± 0.2	2.3 ± 0.1	0.99
30	9.3 ± 1.4	2.4 ± 0.3	
100	25.7 ± 0.1	2.5 ± 0.2	
CGP44530			
10	1.85 ± 0.04	2.6 ± 0.2	1.01
30	3.2 ± 0.2	3.0 ± 0.1	
100	10.7 ± 0.5	3.7 ± 0.4	
CGP38593			
30	2.5 ± 0.1	2.7 ± 0.1	0.95
60	4.1 ± 0.2	2.5 ± 0.4	
100	6.8 ± 0.3	3.0 ± 0.1	

EC₅₀ is the effective dose that activates 50% of the maximal current when tested at ρ_1 receptors expressed in *Xenopus* oocytes. EC₅₀ values are expressed as mean \pm S.E.M. ($n = 3-6$) and are determined by fitting data from individual oocytes using Kaleidagraph 2.1 (1990). EC₅₀ values of GABA have shifted to the right in the presence of a known concentration of the antagonist. $-\log K_B$ values were determined as described in Section 2. The K_B values are shown in Table 1. n_H is the Hill coefficient. These are greater than 1, indicating that more than 1 molecule of GABA is required for the channel to open. Slope of Schild plot analysis indicating competitive antagonism over the tested concentrations.

IC₅₀ of 2.47 ± 0.04 μ M. The methylphosphinic analogue, CGP70523 and phosphinic analogue, CGP70522, of CACA were antagonists with IC₅₀ values of 38.9 ± 4.9 μ M and > 100 μ M respectively. These compounds had lower affinity for GABA_C receptors than the methylphosphinic and phosphinic analogues of GABA and TACA. The order of potency of the methyl phosphinic acids and phosphinic acids is CGP35024 > CGP27492 > CGP44530 > CGP38593 > CGP70523 \gg CGP70522. The new compounds CGP44530, CGP38593, CGP70523 and CGP70522 were weaker at the GABA_C receptor than the existing phosphinic acid, CGP27492 and the methylphosphinic acid, CGP35024.

CGP35024, CGP27492, CGP44530 and CGP38593 were found to be competitive antagonists. The gradients of the Schild regression plots were not significantly different from 1 over the concentrations tested, indicating that these compounds compete for the same site as GABA.

CGP36742 was found to be an antagonist with moderate potency at the GABA_C receptor with an IC₅₀ value of 62.5 ± 0.5 μ M. This compound is orally active showing cognitive enhancement effects. Other related compounds such as CGP35348, CGP46381, CGP51176 and CGP55845A (Fig. 3) are also orally active but do not show cognitive enhancement effects as observed for CGP36742. These were screened at 100 μ M and had no effect as

either agonists or antagonists at GABA_C receptors. These compounds show high selectivity as GABA_B receptor antagonists.

4. Discussion

Selective agonists and antagonists are needed to determine the physiological role of GABA_C receptors. GABA is a flexible compound due to rotation about the C2–C3 and C3–C4 bonds. It can exist in a range of low-energy conformations (Johnston et al., 1978; Allan and Johnston, 1983). Two of these conformations have been restricted by the introduction of unsaturation in the form of a double bond at the C2–C3 position. The two compounds that represent these restricted conformations are CACA and TACA (Johnston et al., 1975). CACA and TACA have fewer degrees of rotational freedom than GABA and can only rotate about the C3–C4 bond (Johnston et al., 1978; Allan and Johnston, 1983). CACA is a partially folded analogue of GABA. It has moderate activity at GABA_C receptors expressed in *Xenopus* oocytes and is to date the most selective, albeit weaker agonist at these receptors having minimal activity on GABA_A and GABA_B receptors (Johnston, 1996b). TACA is an extended analogue of GABA. It has potent agonist activity at GABA_C receptors expressed in *Xenopus* oocytes; however, it is not selective as it is also a potent GABA_A receptor agonist (Johnston, 1996b).

Woodward et al. (1993), using poly(A)⁺ RNA from mammalian retina expressed in *Xenopus* oocytes, tested many GABA_A and GABA_B receptor agonists and antagonists to determine a pharmacological profile for GABA_C receptors. From this study, it was found that the phosphinic and methylphosphinic analogues of GABA, which are known potent GABA_B receptor agonists, were potent antagonists at GABA_C receptors. As a result of the structure-activity relationship study and the selectivity of CACA for GABA_C receptors, we have investigated the methylphosphinic acid and phosphinic acid analogues of CACA and the closely related analogue, TACA, as potential GABA_C receptor antagonists.

The development of many alkylphosphinic and phosphinic analogues of GABA has yielded novel GABA_B receptor agonists and antagonists (Olpe et al., 1990, 1993; Bittiger et al., 1992, 1993; Froestl et al., 1992, 1995a,b), including the methylphosphinic and phosphinic analogues of TACA and CACA, i.e., CGP44530, CGP38593, CGP70522 and CGP70523. In this study, we tested these compounds on GABA_C receptors expressed in *Xenopus* oocytes, and found them to be competitive antagonists. The antagonist potencies of CGP44530, CGP38593, CGP70522 and CGP70523 were found to be lower than that of the methylphosphinic and phosphinic analogues of GABA, CGP35024 and CGP27492.

Table 3

A summary of the affinities of the compounds used in this study at GABA receptors

Compound	Receptor affinity ^a (μM)		
	GABA _A ^a	GABA _B ^c	GABA _C ^d
GABA	0.128 ^k	0.033	EC ₅₀ = 0.82 ^e
CGP27492	1.7 ^k	0.005	2.47
CGP35024	Inactive at 10 ^k	0.016	0.75
CGP36742	508	38	62
TACA	0.14 ^{f,k}	Inactive at 100 ^g	EC ₅₀ = 0.44 ^e
CGP38593	6.8	0.28	7.68
CGP44530	Inactive at 100	0.65	5.53
CACA	25 ^{f,k}	Inactive at 100 ^g	EC ₅₀ = 37 ^e
CGP70522	6.6	4.4	> 300
CGP70523	242	16	38
Isoguvacine	1.4 ^{f,k}	Inactive at 500 ^h	EC ₅₀ = 99 ⁱ
TPMPA	K _b = 320 ^j	EC ₅₀ ~ 500 ^h	K _b = 2.1 ⁱ

^a Receptor affinities are IC₅₀ values unless otherwise stated.

^b IC₅₀ values, i.e., concentration that inhibits 50% of the total [³H]muscimol binding using rat cortical membranes (Froestl et al., 1995a,b).

^c IC₅₀ values for the inhibition of [³H]CGP27492 binding using rat cortical membranes (Froestl et al., 1995a,b).

^d IC₅₀ values for the inhibition of the response of 1 μM GABA using human ρ₁ mRNA expressed in *Xenopus* oocytes as described in Section 2.

^e EC₅₀ values, i.e., the effective dose that activates 50% of the maximal current when tested at ρ₁ receptors expressed in *Xenopus* oocytes as described in Section 2.

^f IC₅₀ values for the inhibition of the total Na-independent [³H]GABA binding using rat brain membranes (Johnston et al., 1978).

^g Data from Kerr and Ong (1995) using guinea-pig ilium, in the presence of bicuculline, against baclofen-depression of twitch contractions.

^h Data from Ragozzino et al. (1996) using whole-cell patch recordings from pyramidal neurons in hippocampal slices in the presence of bicuculline (20 μM).

ⁱ Data from Murata et al. (1996) using human ρ₁ mRNA expressed in *Xenopus* oocytes.

^j Data from Ragozzino et al. (1996) using poly(A)⁺ RNA from rat cortex expressed in *Xenopus* oocytes.

^k EC₅₀ values for GABA, CGP27492, CGP35024, TACA, CACA and isoguvacine using poly(A)⁺ RNA from rat cortex expressed in *Xenopus* oocytes are 107 μM, 938 μM, inactive at 1 mM, 133 μM, inactive at 5 mM, and 305 μM, respectively (Woodward et al., 1993). These values are different from the values obtained from radioligand binding assays.

The relative effects of the compounds at GABA_A, GABA_B and GABA_C receptors are shown in Table 3. The compounds, CGP38593, CGP70522 and CGP27492, were moderately potent at GABA_A receptors when tested using radioligand binding assays (IC₅₀ = 6.8 μM; IC₅₀ = 6.6 μM and IC₅₀ = 1.7 μM, respectively) (Froestl et al., 1995a). However, the compounds were more potent at GABA_B receptors than at GABA_A receptors using this assay. Similarly, these compounds appear more potent at GABA_B receptors than at GABA_C receptors.

The unsaturated methylphosphinic acid analogues of TACA and CACA, CGP44530 and CGP70523, have weak effects at GABA_A receptors when tested using radioligand binding assays (IC₅₀ = > 100 μM and 242 μM, respectively). The methylphosphinic acid analogue of GABA,

CGP35024, showed no effects at 10 μM (Froestl et al., 1995a). This moiety appears to reduce the affinity of the compounds for GABA_A receptors. In contrast, these compounds appear to be more potent at GABA_B receptors than at GABA_A receptors, and appear more potent at GABA_B receptors than at GABA_C receptors. Subsequently, introducing unsaturation to the phosphinic acid and methylphosphinic acid analogues of GABA reduces the potency of the compounds at GABA_B receptors. Consequently, it maybe the combination of the methylphosphinic acid moiety and unsaturation that reduces the apparent selectivity for GABA_B receptors and gains selectivity for GABA_C receptors.

In the above discussion, we have compared the potencies and selectivity of a variety of compounds in which ligand binding assays on GABA_A and GABA_B receptors were compared to electrophysiological studies on GABA_C receptors. However, it is not possible to make a quantitative comparison between receptor types because different techniques have been employed. The potencies, for example of GABA and TACA, are lower when electrophysiological methods are employed compared to radioligand binding assays.

CGP36742 was shown to be a moderately potent antagonist at GABA_B receptors using a [³H]CGP27492 binding assay ($\text{IC}_{50} = 35 \mu\text{M}$) (Bittiger et al., 1992; Olpe et al., 1993; Froestl et al., 1995a). It had weak effects at GABA_A receptors ($\text{IC}_{50} = 500 \mu\text{M}$) (Bittiger et al., 1992) and had no effect at other receptor types including NMDA, benzodiazepine, quisqualate, kainate, muscarinic cholinergic, adrenergic, serotonergic and histaminergic receptors (1 mM) (Bittiger et al., 1992; Froestl et al., 1995b). However, in the present study, CGP36742 showed moderate antagonist activity at GABA_C receptors ($\text{IC}_{50} = 62 \mu\text{M}$) and its apparent selectivity for GABA_B compared to GABA_C receptors was approximately 2-fold. This compound has shown promising therapeutic potential in the treatment of cognitive deficits, petit mal epilepsy and depression (Bittiger et al., 1992). Therefore, it is possible that antagonism of GABA_C receptors contributes to the cognitive enhancement potentiation of CGP36742 that is not shown by other orally active GABA_B receptor antagonists (Froestl et al., 1995b).

TPMPA was recently synthesised and tested at GABA_A, GABA_B, and GABA_C receptors (Murata et al., 1996). It is a conformationally restricted analogue of CGP44530, and the methylphosphinic analogue of isoguvacine. It was found to be more than 100 times selective as an antagonist for the GABA_C receptors than GABA_B receptors and is 500 times more selective at GABA_C receptors than GABA_A receptors (Murata et al., 1996; Ragozzino et al., 1996). The relative lack of selectivity of the methylphosphinic and phosphinic analogues of TACA and CACA may be explained by the rotational freedom about the C3–C4 bond. TACA and its methylphosphinic and phosphinic analogues of these can attain two low-energy conforma-

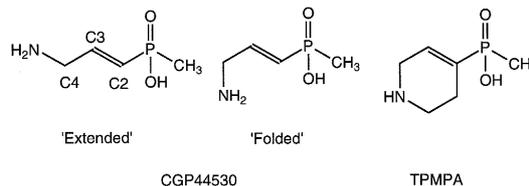


Fig. 4. The introduction of unsaturation in the form of a double bond at the C2–C3 position of CGP35024 restricts rotation at this position. CGP44530 has two low-energy conformations ('extended' or 'folded') that result from rotation about the C3–C4 bond. Restricting rotation about this bond, as with TPMPA, offers a 'folded' conformation that is both potent and selective for the GABA_C receptor. This may explain why CGP44530 was not as selective as TPMPA.

tions about the C3–C4 bond as seen in Fig. 4. Restricting the rotation about the C2–C3 and C3–C4 bonds to one of the conformations, as with compounds like TPMPA, offers a folded conformation that is both potent and selective for the GABA_C receptors compared to GABA_A and GABA_B receptors.

This work has enhanced our understanding of the structural basis for the development of selective agonists and antagonists of the GABA_C receptors. It has helped characterise these receptors by building onto the existing structure-activity relationship trends of phosphinic and methylphosphinic acid analogues of GABA.

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